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Running Head: Pharmacokinetic Modeling for Study Design

Keywords: pharmacokinetic (PK) modeling, study design, exposure, dietary intake, pesticide

Abbreviations:

NRC	National Research Council
MNCPES	Minnesota Children's Pesticide Exposure Study
PK	pharmacokinetic
EPA	United States Environmental Protection Agency
SD	Standard Deviation

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ABSTRACT

Validating an exposure pathway model is difficult because the biomarker, which is often used to evaluate the model prediction, is an integrated measure for exposures from all the exposure routes and pathways. The purpose of this paper is to demonstrate a method to use pharmacokenetic (PK) modeling and computer simulation to guide the design of field studies to validate pathway models. The children's dietary intake model was discussed in detail as an example. Three important aspects were identified for a successful design to evaluate the children's dietary intake model: (1) longitudinally designed study with significant changes in the exposure for the route/pathway of interest; (2) short biological half-life of the selected chemical; and (3) surface loading of the selected chemical at sufficient levels. Using PK modeling to guide a study design allowed a path-specific exposure model to be evaluated using urinary metabolite biomarkers.

INTRODUCTION

Modeling is often the only cost-effective tool for making exposure and risk assessments; however, an evaluation is difficult, especially if it is for a pathway specific model such as a dietary exposure model. A biomarker, such as urinary metabolite, which is often used to evaluate a model prediction, is an integrated measure of exposures from all routes, including inhalation, ingestion, and dermal. Biomarkers also have inherent problems such as large intra- and inter- individual variabilities and unclear metabolic pathways. These uncertainties complicate the interpretation of biomarker measurements relative to the routes responsible for the exposures. Furthermore, the detection limits for urinary metabolite biomarkers are often not low enough to obtain a measurement, resulting in a substantial number of non-measurable observations, which make model validation impossible.

Despite these problems, the demand for model evaluation is increasing (Oreskes, 1998). Biomarkers have been used to evaluate various exposure models, such as a lead exposure model (Zaragoza and Hogan, 1998), a dietary cadmium model (Choudhury et al., 2001), and a dietary methyl mercury intake model (Ponce et al., 1998). In all these studies, however, PK modeling was used to provide interpretations for exposure and biomarker measurement. The potential of PK modeling in guiding a study design for model evaluation was not explored.

One of the problems in children's exposure studies is assessing dietary exposure. Because children touch foods with their hands, excess dietary intake could result from hand-to-food, surface-to-food, and hand-to-surface-to-food contacts in contaminated homes (Melnyk et al., 2000). No direct method to measure this excess exposure is available; therefore, a dietary intake model was developed (Akland et al., 2000).

Because the children's dietary intake model pathway specific, evaluating it has considerable challenges. PK modeling makes the evaluation possible. Unlike other model evaluation efforts, here PK modeling was used to guide the design of a field study to evaluate a pathway specific model using urinary metabolites measured in overnight voids. The children's

dietary intake model for pesticide exposure was used as an example. The principle of using pharmacokinetic modeling for study design, however, should be applicable in other similar cases.

METHODS

Conceptual model

A simplified, single-compartment model that can be used in the design of a field study is shown in Figure 1. In a single compartment model, the body receives exposures from three major routes: inhalation, ingestion, and dermal. The ingestion route receives exposures from two pathways: dietary ingestion and non-dietary ingestion (caused by hand-to-mouth or object-to-mouth activities). The body eliminates the exposure through urine and other biological routes, such as exhaled air, feces, and other bodily fluids.

To demonstrate how a specific pathway model can be evaluated using an overnight urine void, a hypothetical scenario (shown in Figure 2) is presented here. In this hypothetical case, a child receives discrete and varying amounts of dietary exposure, $P_{dietary}$ (μg), from the meals. A child also receives a simplified constant rate for inhalation exposure, $R_{inhalation}$ (μg/min), assuming the child spends most of the time indoors (Lambert et al., 1993). In addition, the child receives a fairly constant non-dietary ingestion exposure, $R_{nondietary}$(μg/min), from hand-to-mouth or object-to-mouth activities that occur when the child is awake during the day. Finally, the child receives a constant rate of dermal exposure, R_{dermal} (μg/min), during the day until he/she is bathed. The exposure amount and rates can be expressed as follows:

$$P_{dietary} = \begin{cases} P_{breakfast} & t = T_1 \\ 0 & T_1 < t < T_2 \\ P_{lunch} & t = T_2 \\ 0 & T_2 < t < T_3 \\ P_{dinner} & t = T_3 \end{cases}$$

$$R_{dermal} = \begin{cases} E_{dermal} & t \le T_4 \\ 0 & t > T_4 \end{cases}$$

$$R_{nondietary} = \begin{cases} E_{nondietary} & t \le T_5 \\ 0 & t > T_5 \end{cases}$$

$$R_{inhalation} = E_{inhalation}$$

Here $P_{breakfast}$, P_{lunch}, and P_{dinner} are the amount of dietary intake from breakfast, lunch, and dinner, respectively, and T_1, T_2, and T_3 are the timing of the meals. E_{dermal} is the rate of dermal exposure before bathing, and T_4 is the time when the child is bathed. $E_{nondietary}$ is the rate of non-dietary exposure before bed, and T_5 is the time when the child goes to the bed. $E_{inhalation}$ is the constant rate of inhalation exposure.

Assuming immediate and 100% absorption through all routes for a single-compartment linear model, the change in the amount of pollutant over time in the compartment can be expressed as follows:

$$\frac{dP_t}{dt} = R_t - kP_t \qquad \text{(Eq. 1)}$$

where P_t is the amount of pollutant in the compartment, k is the first-order biological elimination constant, calculated by $0.693/T_{1/2}$ ($T_{1/2}$ is the biological half-life)(Schoenwald, 2001). R_t is the sum of $R_{inhalation}$, $R_{non\text{-}dietary}$ and R_{dermal}. Dietary exposures from the three meals can be viewed as additional multiple bolus intake at time T_1, T_2 and T_3.

Using the principle of superposition (Schoenwald, 2001), the solution to Equation 1 can be expressed as follows:

$$P_t = P_{breakfast}e^{-k(t-T_1)} + P_{lunch}e^{-k(t-T_2)} + P_{dinner}e^{-k(t-T_3)} + \frac{E_{dermal}}{k}(1-e^{-kT_4})(1-e^{-k(t-T_4)}) + \frac{E_{nondietary}}{k}(1-e^{-kT_5})(1-e^{-k(t-T_5)}) + \frac{E_{inhalation}}{k}(1-e^{-kt}) \quad \text{(Eq. 2)}$$

The amount of pollutant metabolite eliminated into overnight void from 8 p.m. to 8 a.m. is:

$$Y_{overnight} = \alpha k \frac{M_{metabolite}}{M_{polluant}} \int_{8pm}^{8am} P_t dt \quad \text{(Eq. 3)}$$

where α = fraction of pollutant that is eliminated via urine

k = the first-order biological elimination constant

P_t = the amount of pollutant in the compartment

$M_{pollutant}$ = molecular weight of the pollutant

$M_{metabolite}$ = molecular weight of the urinary metabolite.

Applying Equation 2 to Equation 3, the amount of metabolite in overnight urine, $Y_{overnight}$, becomes

$$\begin{aligned} Y_{overnight} = {} & \alpha k \frac{M_{metabolite}}{M_{polluant}} [\int_{8pm}^{8am} (P_{breakfast}e^{-k(t-T_1)} + P_{lunch}e^{-k(t-T_2)} + P_{dinner}e^{-k(t-T_3)})]dt \\ & + \alpha E_{dermal} \frac{M_{metabolite}}{M_{polluant}} \int_{8pm}^{8am} (1-e^{-kT_4})(1-e^{-k(t-T_4)})dt \\ & + \alpha E_{nondietary} \frac{M_{metabolite}}{M_{polluant}} \int_{8pm}^{8am} (1-e^{-kT_5})(1-e^{-k(t-T_5)})dt \\ & + \alpha E_{inhalation} \frac{M_{metabolite}}{M_{polluant}} \int_{8pm}^{8am} (1-e^{-kt})dt \end{aligned} \quad \text{(Eq. 4)}$$

Equation 4 demonstrates that the amount of metabolite in overnight urine is an additive result of exposure from dietary ingestion, non-dietary ingestion, inhalation, and dermal exposure. Therefore, if we design a study in which exposure from a specific route is varied while exposures from other routes remain the same, we will be able to investigate the exposure through this particular route. For example, if we only alternate daily dietary exposure status, i.e., let the subject have dietary exposure on "dietary exposure day" (when dietary exposures are $P_{breakfast}$, P_{lunch}, and P_{dinner}) followed by "no dietary exposure day" (when dietary exposures $P_{breakfast}$ = P_{lunch}=P_{dinner}=0) and let the exposures from other routes/pathways remain the same, then the difference of the urinary metabolites between these two days is only a function of dietary exposure because exposures from other routes/pathways can be cancelled out. The following equation shows the difference in the amount of urinary metabolites measured in overnight voids after the dietary exposure day and the no dietary exposure day:

$$\begin{aligned}\Delta Y &= Y_{\text{overnight void after exposure day}} - Y_{\text{overnight void after non-exposure day}} \\ &= \alpha k \frac{M_{metabolite}}{M_{pollutant}} \int_{8pm}^{8am} (P_{breakfast} e^{-k(t-T_1)} + P_{lunch} e^{-k(t-T_2)} + P_{dinner} e^{-k(t-T_3)}) dt \end{aligned} \quad \text{(Eq. 5)}$$

Equation 5 indicates that if ΔY, the metabolite difference between overnight voids after the dietary exposure day and the no dietary exposure day, is large enough to be measured, it can be used to evaluate dietary exposure differences on these days. It also indicates that to make the evaluation possible, the dietary exposures on the dietary exposed day also need to be large; the biological half life of the chemical, $T_{1/2}$, needs to be short, as k is proportional to $1/T_{1/2}$; and, a substantial fraction of the metabolites should be eliminated through the urinary pathway.

In reality, however, dietary exposure is hardly zero on the dietary exposure days, because pesticide residues in foods are inevitable. Nontheless, with a careful design, the pesticide residue can be cancelled out and the strategy can still be used, as demonstrated in the following evaluation of the children's dietary intake model.

Children's dietary intake model

The major problem of assessing children's dietary exposure is that young children often touch foods with their hands prior to consumption, thereby increasing contamination of the food and their intake of contaminants through the diet (Melnyk et al., 2000). Because direct methods for sampling the foods as they enter the mouths of young children are not available, a deterministic dietary intake model was developed (Akland et al., 2000). In this model, three terms are considered. They are: (1) the original contaminant residue on the food before handling (Term 1); (2) surface-to-food contamination as the food comes in contact with contaminated surfaces (Term 2); and (3) surface-to-hand-to-food contamination as the child touches the contaminated surfaces and then handles and eats foods (Term 3). Term 1 has also been referred to as "direct dietary ingestion," and Term 2 and Term 3 as "indirect dietary ingestion", respectively. In this model, it is assumed that the activity parameters ($A_{S/F}$, $A_{H/F}$, and $A_{S/H}$) are determined by food types and individual child; and transfer efficiencies ($T_{S/F}$, $T_{H/F}$, and $T_{S/H}$) are determined by food types, surface types, and the chemical properties of the contaminants.

Details of the children's dietary intake model have been discussed previously (Akland et al., 2000). The following is the model for a specific food item consumed after multiple touches by hands and/or surfaces.

$$\begin{aligned} P_{food} &= \text{Term 1} + \text{Term 2} + \text{Term 3} \\ &= UW_T + \sum_{surfaces} C_S F_S T_{S/F} A_{S/F} + \sum_{hands} (C_S T_{S/H} A_{S/H})(T_{H/F} A_{H/F} H_S PH) \end{aligned} \quad \text{(Eq. 6)}$$

where, assuming the pollutant of interest is a pesticide,

P_{food} = Dietary intake of a pesticide for one food (μg)

U = Pesticide residue concentration (μg pesticide/g food)

W_T = Total amount of the individual food consumed (g)

C_S = Loading of the contaminant on the surface (μg pesticide/cm^2)

F_s = Food surface area that comes in contact with the contaminated surface (cm^2)

$T_{S/F}$ = Surface-to-food mass transfer efficiency (dimensionless)

$A_{S/F}$ = Surface-to-food contact frequencies

$T_{S/H}$ = Surface-to-hand mass transfer efficiency (dimensionless)

$A_{S/H}$ = Surface-to-hand contact frequencies

$T_{H/F}$ = Hand-to-food mass transfer efficiency (dimensionless)

$A_{H/F}$ = Hand-to-food contact frequencies

H_s = Total hand surface area (cm^2)

PH = Proportion of hand surface area in contact with contaminated food.

Total dietary exposure for a meal is therefore,

$$P_{meal} = \sum_{\text{all foods}} P_{food} \quad \text{(Eq. 7)}$$

Laboratory experiments have demonstrated measurable surface-to-food, surface-to-hand, and hand-to-food pesticide transfers (Akland et al., 2000; Edwards and Lioy, 1999). Using the children's dietary intake model Equation 6, it was estimated that the extra pesticide intake resulting from young children's eating behaviors, Term 2 and Term 3, could account for up to 80% of total dietary intake if the surface loading of pesticide residue is $5ng/cm^2$ or higher (Akland et al., 2000).

If proved, this result would have profound implications in pesticide regulation and exposure mitigation. However, as shown in Equation 6, the model prediction was based upon the estimation of food surfaces, the surface pesticide loading, the transfer efficiencies, and observation of children's eating behaviors. A natural question for the model prediction is: is the model estimation reasonable?

Using Pharmacokinetic Modeling to Design a Field Study - Children's Dietary Intake Model as an Example

General Concept for Design

The children's dietary intake model is a pathway model. Exposures from other routes/pathways (e.g., non-dietary ingestion, inhalation, and dermal exposure) also contribute to the total urinary pesticide metabolite measurements. Therefore, using urinary biomarker

measurements to evaluate the dietary intake model is difficult. To circumvent the problem, the strategy demonstrated in Equation 5 can be followed, as outlined below.

According to the children's dietary intake model Equation 6, the dietary exposure consists of three terms: residue in food before handling (Term 1), surface-to-food transfer (Term 2), and surface-to-hand-to-food transfer (Term 3). On a day when the child is allowed to eat in an unrestricted normal setting, the child receives environmental exposures through inhalation, dietary ingestion, non-dietary ingestion, and dermal exposure, and the dietary exposure includes Term 1+ Term 2 + Term3. Suppose we restrict a child with clean hands to a clean area and require the same foods to be eaten as on the normal day, then Term 2 + Term 3 are artificially forced to be approximately zero and only Term 1 remains. For the convenience of discussion, the day when the child is restricted to a clean area with clean hands is referred to as "non-exposed day," and the day when the child is allowed to eat at regular places with uncleaned hands is referred to as "exposed day," henceforward. Note on the non-exposed day, the child still receives inhalation, non-dietary ingestion, and dermal exposures. On both the exposed day and the non-exposed day, the child receives the same Term 1 because the same foods are eaten on both days. The exposures the child does not receive on the non-exposed day are the surface-to-food transfer (Term 2) and surface-to-hand-to-food transfer (Term 3).

Theoretically, if inhalation, non-dietary ingestion, and dermal exposures can be kept approximately the same on the exposed day and the non-exposed day, then according to Equation 5, the difference in the amount of urinary metabolites in overnight voids after the exposed day and the non-exposed day is a function of Term 2 and Term 3:

$$\Delta Y = \alpha k \frac{M_{metabolite}}{M_{pollutant}} \int_{8pm}^{8am} ((Term2 + Term3)_{breakfast} e^{-k(t-T_1)} + (Term2 + Term3)_{lunch} e^{-k(t-T_2)} + (Term2 + Term3)_{dinner} e^{-k(t-T_3)}) dt \quad \text{(Eq. 8)}$$

Compared to Equation 5, Term 1 has been cancelled out because the child's diet is restricted so that the same foods were eaten on the exposed day and the non-exposed day. An effective method to maintain the same exposure on the exposed day and the non-exposed day for other exposure

routes/pathways while alternating the exposure for the pathway of interest is to conduct the study longitudinally so that data from several exposed day/non-exposed day pairs can be collected from the same subjects. This way the participant can serve as his or her own control so that and k can be assumed to be the same variable and behavior pattern variations can be kept at a minimum.

Computer Simulation

Equation 5 and Equation 8 demonstrate how, in theory, a route/pathway exposure model can be evaluated with a study design using metabolites in overnight urinary voids where the exposure status of the route/pathway of interest is varied while the exposures from the other routes/pathways are kept the same. For field studies, the following questions are the keys for study design:

- How long should the half-life of a selected pesticide be?
- What is the minimum level of surface pesticide loading to produce a measurable metabolite concentration in the overnight void?
- What is the minimum level of surface pesticide loading to make indirect dietary ingestion a measurable quantity in overnight urine?
- Will exposures from other pathways "mask" the exposure caused by surface-to-food and surface-to-hand-to-food transfer?
- How large of a sample size is needed?

An important assumption for the analytical solutions, Equation 5 and Equation 8, is that exposures from inhalation, non-dietary ingestion, and dermal remain constant. In reality, however, this may not be true. To investigate whether a varying inhalation/non-dietary/dermal profile will mask the urinary metabolite difference caused by dietary exposure, which is the key to the study design, we need to let the exposure rates vary across time. To demonstrate, however, we only set non-dietary ingestion exposure to vary across time because of its significance (Zartarian, 2000). Inhalation and dermal exposures remained constant.

The varying exposure rates make it impossible to use analytical solutions Equation 5 and Equation 8. Therefore, a computer simulation was conducted to answer the above questions needed for a field study. To conduct the computer simulation, all the input parameters were set at values for a likely scenario based upon published literature. The parameters of interest were then varied (one at a time) to observe their impact on the output variable (i.e., urinary metabolite concentration). Computer simulation was based upon numerical solution to Equation 3 using Euler's method:

$$\begin{aligned} P_t &= P_{t-1} + (dP_t / dt) \times \Delta t \\ &= P_{t-1} + (R_{nondietary} + R_{inhalation} + R_{dermal}) \times \Delta t + P_{dietary} - kP_{t-1} \times \Delta t \end{aligned} \quad \text{(Eq. 9)}$$

Details of the estimation/simulation of the exposure rates are given below.

Inhalation exposure rate. Exposure via inhalation per hour was estimated as follows:

$$R_{inhalation} = LV \quad \text{(Eq. 10)}$$

where L is the air concentration (ug/L) and V is the ventilation rate for children (L/hr).

Non-dietary ingestion exposure rate. The non-dietary ingestion exposure rate mentioned here is the exposure incurred when children put contaminated hands or toys into their mouth. To simulate the varying profile, the time that a child is awake (assuming from 8:00 a.m. to 8:00 p.m.) was divided into equal time intervals. The non-dietary exposures received in these time intervals were assumed to be normally distributed. The mean of the $R_{nondietary}$ was calculated by the following formula:

$$\text{Mean of } R_{nondietary} = H_s P H_m C_h F r_{h/m} \quad \text{(Eq. 11)}$$

where

H_s = Total hand/toy surface area (cm^2)

PH_m = Proportion of total hand/toy surface area coming in contact with mouth

C_h = Surface loading of the contaminant on the hand /toy (µg pesticide/cm^2)

$Fr_{h/m}$ = Frequency of mouthing activity during the time interval.

Using published data, we estimated a mean of 0.0267µg/min for $R_{nondietary}$. A standard deviation of 0.0179 ug/min was assumed so that more than 50% of the simulated values were within one standard deviation (Table 1). Because non-dietary ingestion exposure was unlikely when the child is asleep, we assumed zero non-dietary exposures between 8:00 p.m. and 8:00 a.m. The simulation of $R_{nondietary}$ for a 1 minute time interval can be summarized in the following formula:

Dermal exposure. We ignored dermal exposure in the computer simulation for two reasons. First, diazinon (which was the pesticide of interest) exposure through skin absorption has been reported in the literature to be minimal, although this may not be the case for other chemicals. Using radiolabeled diazinon in an acetone solution or lanolin grease on the forearm or abdomen, Wester and colleagues (Wester et al., 1993) reported a total of only 2.2% skin absorption over 24 hours. Second, the purpose of the study was to guide study design rather than to establish a definitive relationship between exposure and metabolites.

Applying Equation 12, Equation 10, and Equation 7 to Equation 9, the model used to conduct the computer simulation was obtained.

Table 1 lists the parameters used to estimate inhalation and non-dietary intake.

Parameters for the children's dietary intake model were obtained from a previous study (Akland et al., 2000). Table 2 demonstrates how to use the children's dietary model to estimate exposure for three example foods: cheerios, apple, and tortilla. In these examples, the pesticide residue was assumed to be 6 ng/g for all foods (NRC, 1993). Parameters $T_{S/H}$, $A_{S/H}$, $T_{H/F}$, $A_{H/F}$, and PH were also estimated from the previous study (Akland et al., 2000). Because cheerios are normally eaten with utensils, only Term1 is calculated for total dietary ingestion. Apple and

tortilla, however, were estimated for Term 2 and Term 3, as these foods are normally handled by children. Other foods used to estimate a hypothetical child's exposed day's total dietary intake included rice (two tablespoons), chicken nuggets (4 pieces), and ham (1 slice). On the following unexposed day, only Term 1 from the foods remained, and Term 2 and Term 3 were assumed to be zero. The examples shown in Table 2 demonstrate that by varying surface loading, different pesticide transfers are obtained. Therefore, the minimum level of surface pesticide loading to make indirect dietary ingestion a measurable quantity in overnight urine can be estimated.

Computer simulation was conducted using Microsoft Excel 2002. Equations for calculating $R_{inhalation}$, $R_{non\text{-}dietary}$, and $P_{dietary}$ were keyed in, and variables of interest, such as biological half-life, dust loading, air concentration, and non-dietary intake, were set in such a way so that they could be easily varied to conduct the simulation. The simulation results were also plotted using Microsoft Excel.

Sample size calculation

Once the results from the simulation were obtained, sample size was calculated based upon a one-sided t-test of hypothesis: $Y_{overnight\ void\ after\ exposed\ day} = Y_{overnight\ void\ after\ non\text{-}exposed\ day}$ vs $Y_{overnight\ void\ after\ exposed\ day} > Y_{overnight\ void\ after\ non\text{-}exposed\ day}$ (Kleinbaum et al., 1988).

RESULTS

Urinary Measurements and Biological Half-life

Figure 3 at the end of this report shows the urinary metabolite measurements in overnight voids as point estimates (when the urine samples are collected at 8:00 a.m.) after three exposed day/non-exposed day pairs with various lengths of biological half-life of the selected chemical. The results indicated that the success of the validation is heavily dependant on the biological half-life of the chosen chemical. If the chemical has a relatively short half-life, such as for malathion (3 to 4 hrs) (Lyon et al., 1987) or diazinon (~6 hrs) (Iverson et al., 1975), it is possible to detect a change in the urine metabolite concentration. The amount in the plasma also returns to nonexposed levels, which makes the evaluation of the model possible. However, if the biological

half-life is longer than 16 hours, a large sample size is required because the difference between urinary metabolites after exposed days and non-exposed days becomes small and the amount in the plasma is carried over from day-to-day with no recovery. When the biological half-life is as long as or longer than 27 hrs (such as chlorpyrifos), the chance of successful validation using the exposed/non-exposed day design is even smaller because there is minimal difference in the urinary metabolite concentrations. Nonetheless, an alternative design, such as one exposed day followed by two non-exposed days to let the body further eliminate the metabolites, might be possible. This alternative design, however, substantially increases field difficulties because on the two non-exposed days the field team would need to ensure no Term 2 and Term 3 intake occurs.

Pesticide Loading

Surface pesticide loading is the source for surface-to-food and surface-to-hand-to-food transfer. Results of variations in the surface loading and urinary metabolites for a compound with a biological half life of 8 hours are shown in Figure 4. The results indicate that even if the chemical's half life is short, a preferable loading of 4 ng/cm^2 or above is still needed to generate observable differences in urinary metabolites in the overnight voids after the exposed day and the non-exposed day. This level of loading can be found after indoor pesticide application (Byrne et al., 1998). However, when the loading decreases to 1 ng/cm^2 or less, it is very difficult to see the differences in the urinary metabolite amount in overnight voids after exposed and non-exposed days. In the Minnesota Children's Pesticide Exposure Study (MNCPES), the mean surface chloropyrifos loading measured by a surface press ranged from 0.03 to 32.6 ng/cm^2, with a mean of 0.48 ng/cm^2 (Lioy et al., 2000). These results answer the question about exposure scenario, i.e. households with surface pesticide loading >4ng/cm^2 are preferred for efficient design and houses that have frequent indoor pesticide applications are most likely to meet the criterion.

Impact of Exposure from Other Routes/Pathways

Non-dietary ingestion pathway. Figure 5 attempts to answer whether exposure from non-dietary ingestion will mask the dietary exposure and interfere with the validation process. As shown in the figure, when non-dietary ingestion exposure is normally distributed with a mean of 1.6 □g/hr (SD=1.1 □g/hour), the mask effect is small enough to allow the biomarker differences caused by dietary exposure difference to be observed. However, when non-dietary ingestion

exposure reaches a mean of 3.2 ug/hr, the mask effect becomes obvious because the difference in urine metabolite concentrations becomes small and inconsistent. The 1.6 ug/hour non-dietary ingestion exposure was calculated by assuming a mouthing frequency of 10/hour (Zartarian et al., 1997); (Reed et al., 1999), which was high compared to the current EPA default (Reed et al., 1999) and for each event the child mouths a 40 cm^2 surface (hand or toy) with a relatively high pesticide loading of 4 ng/cm^2. Because these assumptions reflect high-end exposure, we can safely assume that the average level of non-dietary activity will not significantly interfere with the model validation process. Nonetheless, to conduct a successful study, the subjects selected into the study would preferably be children who do not have frequent mouthing activities, such as thumb sucking.

Inhalation route. Similarly, the effect of inhalation exposure (Figure 6) was estimated. The results indicate that inhalation exposure does not cause a large effect on the biomarker differences, even when the hypothetical air concentration was increased to 5 ug/m^3, a level only seen immediately following indoor pesticide application (Akland et al., 2000).

Sample Size

Based upon a pesticide with a biological half-life of 8 hours and assuming a variance of 2 due to measurement errors, a minimum sample size of five pairs of the exposed day and the non-exposed day would be required in homes with pesticide loading of $4ng/cm^2$ or higher to achieve a power of 80% for detecting 3 μg urinary metabolite differences.

DISCUSSION

Evaluating a pathway model is difficult because the biomarker measurements also have contributions from other exposure routes/pathways. Here we demonstrate that a thoughtful design guided by PK modeling can make the evaluation possible. The computer simulation for the children's dietary intake model indicated three important aspects for a successful design: longitudinal design of the study, short half-life of the selected chemical, and high pesticide surface loading. Under normal circumstances, inhalation and non-dietary ingestion exposure would not

mask the dietary exposure as long as they can be kept nearly constant for the non-exposed day and the exposed day.

Using the results from the computer simulation, we selected diazinon and conducted a study with three children in homes with surface loading of > 4ng/cm^2. Each child was followed for at least 6 days, yielding three or more non-exposed day/exposed day pairs. The study results (to be reported in a companion paper) indicated that this design was successful. Using PK modeling as a guidance, field efforts to collect data to evaluate the model can be well planned, and the cost can be substantially reduced.

In this paper, a single-compartment PK model was used. The single-compartment model may not be as accurate as a multi-compartment PK model in prediction, but it has a practical advantage—only two parameters are essential to build a model: the biological half-life of the chemical and the proportion of the chemical eliminated in overnight void. In many cases, these parameters are the only information one can obtain from the literature. Because of this practical advantage, the single-compartment model was recently used again by other researchers to assess pesticide exposure based on urinary biomarkers (Rigas et al., 2001). It should be emphasized that the purpose of this modeling approach is to provide a guidance for the design of field studies. Therefore, it is perhaps not necessary to expend large efforts to develop a complicated model at the front end of the study design. Our field study also indicated that the single-compartment model was adequate for designing the model evaluation study we had conducted.

This paper demonstrated the case of designing a study to appropriately capture data in order to evaluate a dietary exposure model. However, we envision a similar strategy could be used in other cases, such as the non-dietary ingestion exposure model, the dermal exposure model, or the inhalation exposure model.

NOTATIONS

η	fraction of pollutant that is eliminated through urine
$A_{H/F}$	hand-to-food contact frequencies
$A_{S/F}$	surface-to-food contact frequencies

$A_{S/H}$	surface-to-hand contact frequencies
$A_{H/M}$	hand(toy)-to-mouth contact frequencies
C_A	air concentration (µg/L)
Fs	food surface area that comes in contact with the contaminated surface (cm^2)
H_S	total hand surface area (cm^2)
k	first-order elimination rate constant
L_H	loading of contaminant on hand/toy (µg contaminant/cm^2)
Ls	loading of contaminant on surface (µg contaminant/cm^2)
$M_{metabolite}$	molecular weight of urinary metabolite
$M_{pollutant}$	molecular weight of pollutant compound
$P_{breakfast}$	amount of pollutant in breakfast (µg)
P_{lunch}	amount of pollutant in lunch (µg)
P_{dinner}	amount of pollutant in dinner (µg)
P_{food}	amount of pollutant in one food (µg)
P_{meal}	amount of pollutant in one meal (µg)
$P_{dietary}$	amount of dietary exposure received from all meals (µg)
PH	proportion of hand surface area in contact with contaminated food
Pt	amount of pollutant in the compartment (µg)
R_{dermal}	dermal exposure rate (µg/hr)
$R_{inhalation}$	inhalation exposure rate (µg/hr)
$R_{nondietary}$	non-dietary ingestion exposure rate (µg/hr)
T_1	Timing for breakfast
T_2	Timing for lunch
T_3	Timing for dinner
T_4	Timing for bath
T_5	Timing when child goes to bed
$T_{H/F}$	hand-to-food transfer efficiencies
$T_{S/F}$	surface-to-food transfer efficiencies
$T_{S/H}$	surface-to-hand transfer efficiencies
U	pollutant residue in food (µg/g)

V	ventilation rate for children (L/hr)
Wt	total amount of food consumed (g)
$Y_{overnight}$	amount of urinary metabolite in overnight void
$Y_{\text{overnight void after exposure day}}$	amount of urinary metabolite in overnight void after exposed day
$Y_{\text{overnight void after non-exposure day}}$	amount of urinary metabolite in overnight void after non-exposed day

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Table 1. Parameters for Inhalation and Non-Dietary Ingestion Exposures

	Type of distribution used in simulation	Variables
Inhalation exposure	Constant	V=4.2 L/min C=0.5 μg/m^3 (Byrne et al., 1998)
Non-dietary exposure	Normal distribution with Mean=0.0267 μg/min and SD=0.1795μg/min for 8:00 a.m.-8:00 p.m.; 0 for 8:00 p.m. to 8:00 a.m.	H_s=200 cm^2 PH_m = 0.2 C_h= 4 ng/cm^2 (Byrne et al., 1998; Lu and Fenske, 1999) $Fr_{h/m}$= 10 /hr (Reed et al., 1999; Zartarian et al., 1997)

Table 2. Parameters Used to Calculate Dietary Intake from Cheerios, Apple, and Tortilla (Akland et al., 2000)

Cheerios	(half bowl)		
Term 1[a]			
R	0.006 µg/g		
F_T	30 g	Term1= 0.18µg	
		$P_{breakfast}$=Term1= 0.18µg	
Apple*	(1/3 apple)		
Term 1			
R	0.006 µg/g		
F_T	80 g	Term1= 0.48	
Term 2[b]			
F_S	100 cm2		
C_S	0.004 µg/cm2		
$T_{S/F}$	0.5		
$A_{S/F}$	1	Term2= 0.2	
Term 3[c]			
C_S	0.004 µg/cm2		
$T_{S/H}$	0.4		
$A_{S/H}$	10		
$T_{H/F}$	0.03		
$A_{H/F}$	10		
H_S	200 cm2		
PH	0.9	Term3= 0.86	
		P_{lunch}=Term1+Term2+Term3=	1.54
Tortilla	(half of a tortilla)		
Term 1			
R	0.006 µg/g		
F_T	65 g	Term1= 0.39	
Term 2			
F_S	200 cm2		
C_S	0.004 µg/cm2		
$T_{S/F}$	0.5 (chair-food)		
$A_{S/F}$	1	Term2= 0.4	
Term 3			
C_S	0.004 µg/cm2		
$T_{S/H}$	0.5		
$A_{S/H}$	20		
$T_{H/F}$	0.03		
$A_{H/F}$	20		
H_S	200 cm2		
PH	0.9	Term3=	4.32
		P_{dinner}=Term1+Term2+Term3= 5.11	

* Using model equation (10) to estimate dietary intake for apple:
Term 1= 0.006 (µg/g) × 70 (g)=0.42 µg
Term 2=100 (cm^2) × 0.004 (µg/cm^2) × 0.5 × 1=0.2 µg
Term3=0.004 (µg/cm^2) × 0.4 × 10 × 0.03 × 10 × 200(cm^2) × 0.9=0.86 µg

Figure Legends

Figure 1. Single-compartment model for exposures from different pathways.

Figure 2. Exposure functions for a hypothetical child.

Figure 3. Effect of biological half-life on urinary measurements in the non-exposed day/ exposed day design.

Figure 4. Effect of surface loading on urinary metabolite measurements in the non-exposed day/exposed day design.

Figure 5. Effect of non-dietary exposure on urinary measurements in the non-exposed day/exposed day design.

Figure 6. Effect of inhalation exposure on urinary metabolite measurements in the non-exposed day/exposed day design.

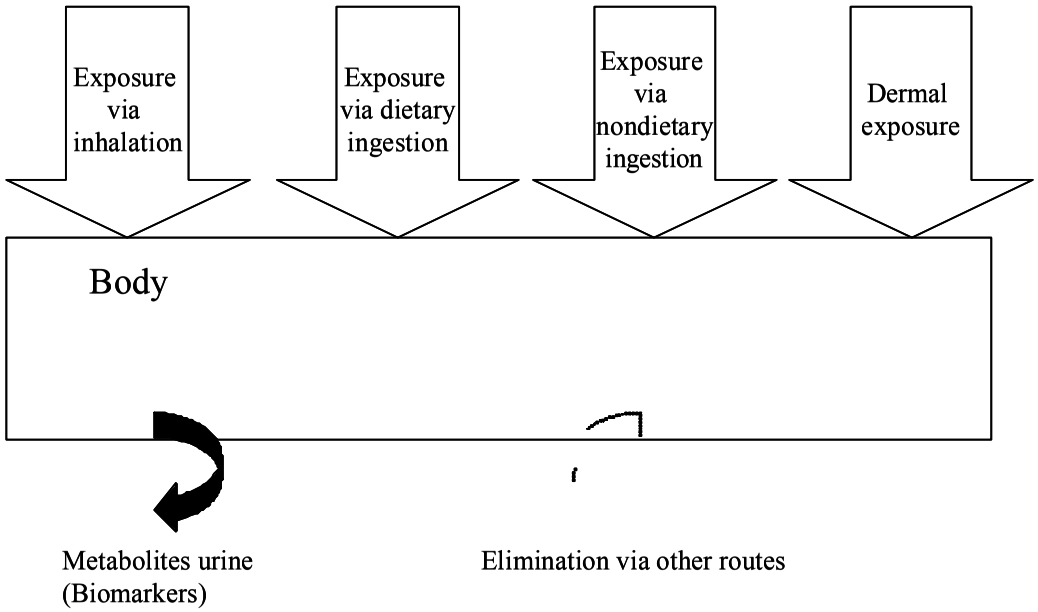

Figure 1. Single compartment model for exposures from different pathways

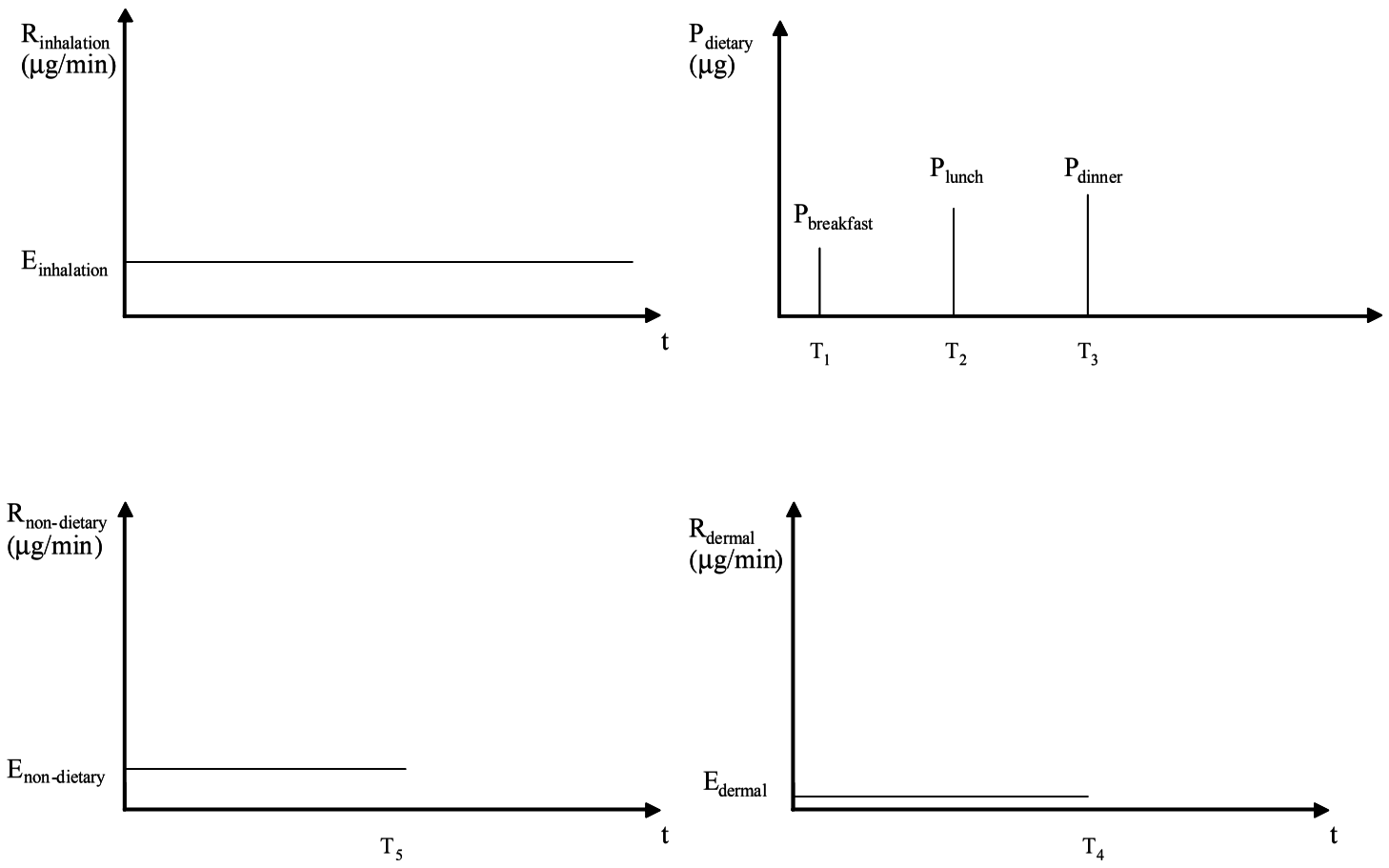

Figure 2. Exposure functions for a hypothetical child

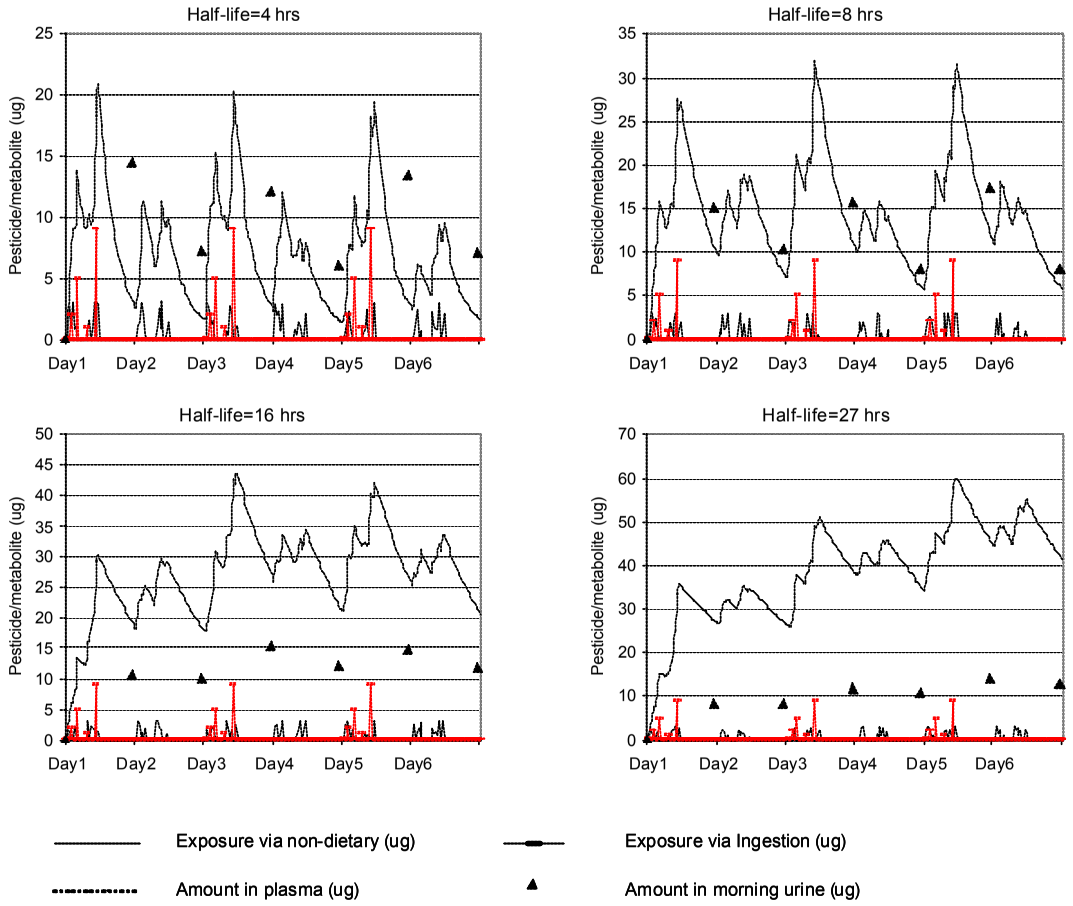

Figure 3. Effect of biological half-life on urinary measurements in the non-expose day/expose day design

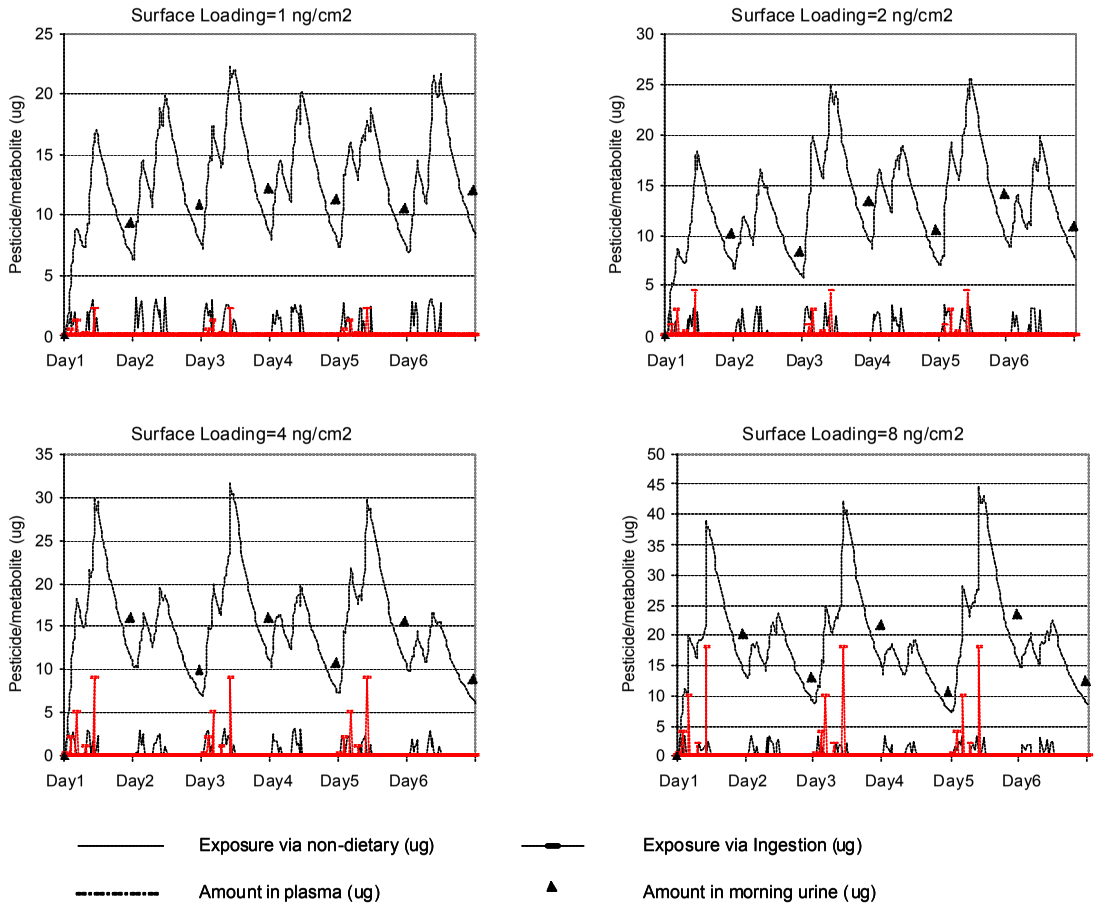

Figure 4. Effect of surface loading on urinary measurements in the non-expose day/expose day design

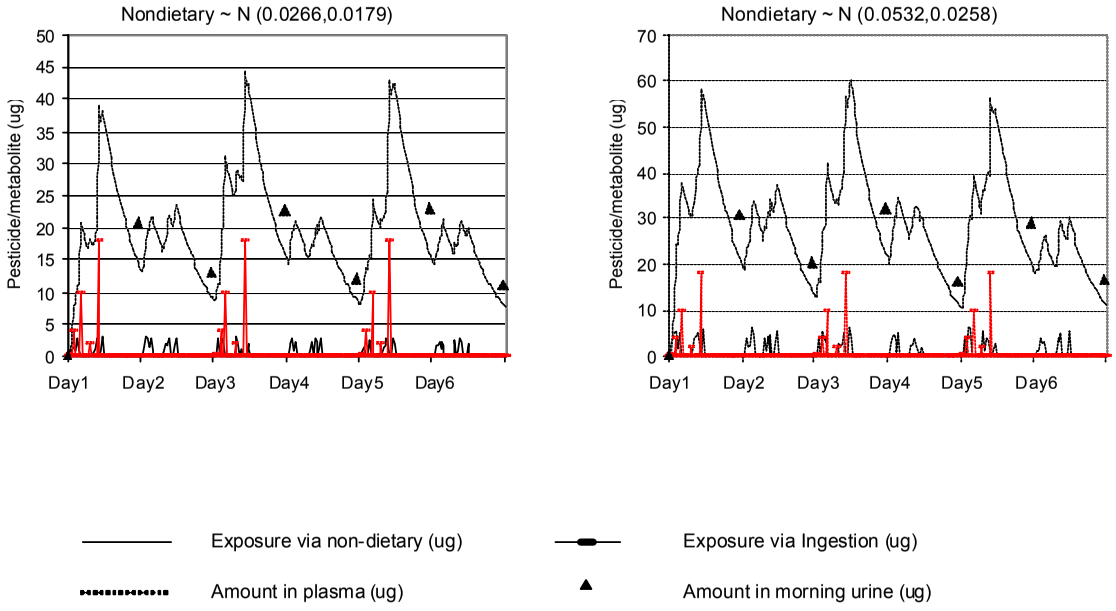

Figure 5. Effect of nondietary exposure on urinary measurements in the non-expose day/expose day design

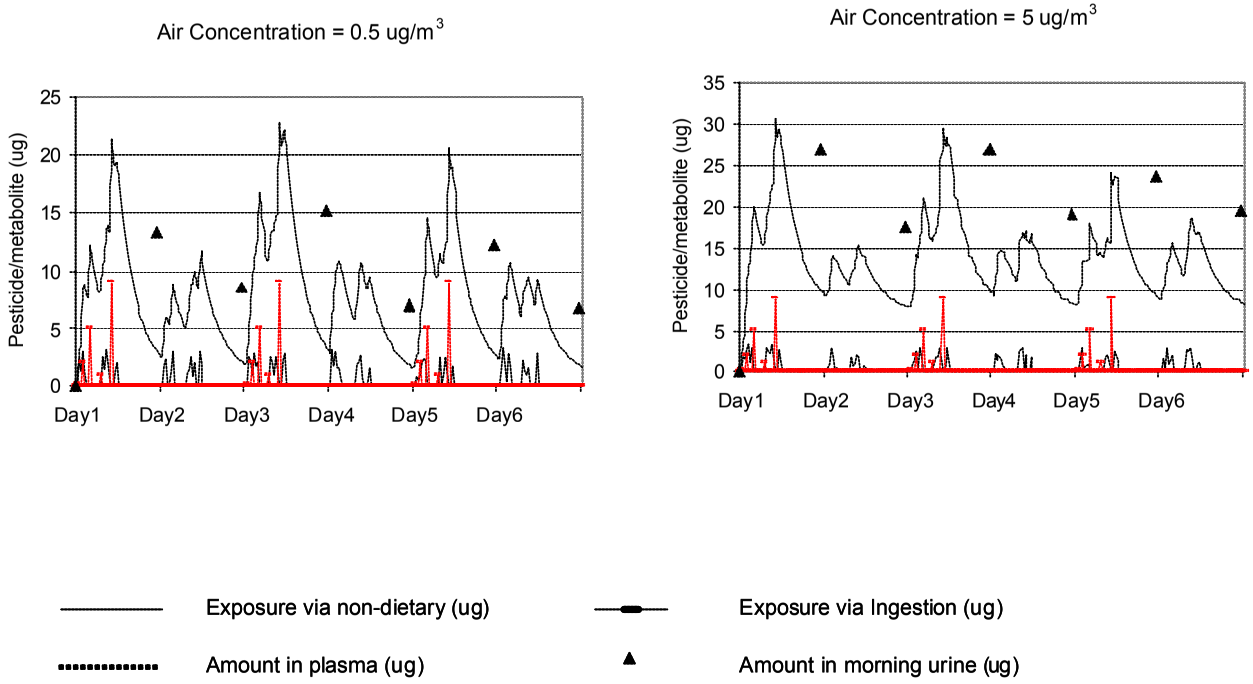

Figure 6. Effect of inhalation on urinary measurements